Amendment Dated: November 12, 2008 Reply to Office Action of July 8, 2008

Examiner: Taeyoon Kim

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (withdrawn-currently amended) A method of culturing human embryonic stem (ES) cells with reduced differentiation comprising:

growing the human ES cells in culture, the cells proliferating in an unconditioned culture medium on a flexible solid porous matrix without conditioned media and in the absence of fibroblast feeder cells in an apparatus configured to apply periodic strain to the matrix and the human ES cells and

b) applying an effective amount of periodic strain on the flexible matrix to stretch the matrix and the human ES cells thereon, such that more of the human ES cells in the culture medium are undifferentiated proliferate and exhibit reduced differentiation relative to human ES cells not subjected to periodic strain.

2. -3. canceled.

- 4. (withdrawn) The method of Claim 1 wherein the cell differentiation is eliminated.
- 5. (withdrawn) The method of Claim 1 wherein the cells are grown on MatrigelTM using BioFlex® untreated culture plates.
- 6. (withdrawn) The method of Claim 1 wherein the cells are grown without the presence of cross-species biological material.
 - 7. (withdrawn) The method of Claim 1 wherein the flexible matrix is MatrigelTM.
 - 8. (withdrawn) The method of Claim 1 wherein the strain is mechanically produced.

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9. (withdrawn) The method of Claim 1 wherein the flexible matrix is stretched using vacuum pressure.

10. (withdrawn) The method of Claim 1 wherein the strain exerted on the flexible matrix is at least about 5%.

11. (withdrawn) The method of Claim 1 wherein the flexible matrix undergoes at least about 6 stretches per minute.

12. (withdrawn) The method of Claim 1 wherein the mechanical strain is from oscillatory stretching of the flexible matrix surface.

13. (currently amended) A cell culture composition comprising:

proliferating human embryonic stem (ES) cells in culture, the cells proliferating in an unconditioned culture medium on a flexible solid porous matrix without fibroblast feeder cells in an apparatus configured to apply periodic strain to the matrix and the human ES cells, wherein more of the human ES cells in the culture medium are undifferentiated than in an otherwise comparable cell culture composition comprising an apparatus not configured to apply periodic strain to the matrix and the human ES cells.

14.-15. canceled.

- 16. (previously amended) The culture composition of Claim 13 wherein substantially all of the human ES cells in the culture are undifferentiated.
- 17. (previously amended) The culture composition of Claim 13 wherein the matrix comprises MatrigelTM and the apparatus comprises a BioFlex® untreated culture plate.

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18. (previously amended) The culture composition of Claim 13 wherein the culture medium is free of cross-species biological material.

- 19. (previously amended) The culture composition of Claim 13 wherein the matrix comprises MatrigelTM.
- 20. (previously amended) The culture composition of Claim 13 wherein the apparatus is configured to apply mechanical strain to the matrix and the human ES cells.
- 21. (previously amended) The culture composition of Claim 13 wherein the apparatus is configured to apply vacuum pressure to the matrix and the human ES cells.
- 22. (previously amended) The culture composition of Claim 20 wherein the mechanical strain comprises oscillatory stretching.
- 23. (previously amended) The culture composition of Claim 13 wherein the apparatus is configured to exert at least about 5% strain on the matrix.
- 24. (previously amended) The culture composition of Claim 13 wherein the apparatus is configured to stretch the matrix at least about 6 times per minute.
- 25. (withdrawn-currently amended) A method of culturing human embryonic stem (ES) cells with reduced differentiation comprising:
- a) growing the human ES cells in culture, the cells proliferating in an unconditioned culture medium on a flexible solid porous matrix without conditioned media and in the absence of fibroblast feeder cells in an apparatus configured to apply periodic strain to the matrix and the human ES cells; and
- b) applying an effective amount of periodic strain on the human ES cells, such that the human ES cells proliferate and exhibit reduced differentiation relative to human ES cells not subjected to periodic strain.

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- 26. (previously amended) The culture composition of Claim 13, wherein the human ES cells are characterized by positive expression of Oct4 and SSEA-4 cell surface markers.
- 27. (previously amended) The culture composition of Claim 26, wherein the human ES cells are immuno-positive for alkaline phosphatase.
- 28. (withdrawn currently amended) A method of culturing undifferentiated human stem cells with reduced differentiation comprising:
- a) growing the undifferentiated human stem cells in culture on a flexible solid porous matrix without conditioned media and in the absence of fibroblast feeder cells <u>in an apparatus configured to apply periodic strain to the matrix and the human ES cells</u>, wherein the stem cells are defined by the positive expression of Oct4 and SSEA-4 cell surface markers; and
- b) applying an effective amount of periodic strain on the flexible matrix to stretch the matrix and the undifferentiated stem cells thereon, such that the undifferentiated cells proliferate and exhibit reduced differentiation relative to undifferentiated human stem cells not subjected to periodic strain.